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Sub-lethal UV radiation during early life stages alters the behaviour, heart rate and oxidative stress parameters in zebrafish (*Danio rerio*)

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ABSTRACT

Environmental UV radiation in sufficient doses, as a possible consequence of climate change, is potent enough to affect living organisms with different outcomes, depending on the exposure life stage. The aim of this project was to evaluate the potentially toxic effects of exposure to sub-lethal and environmentally relevant doses of UVA (9.4, 18. 7, 37.7 J/cm^2) and UVB radiation (0.013, 0.025, 0.076 J/cm^2) on the development and behaviour in early life stages (4.5–5.5 h post fertilization, hpf) of the zebrafish (*Danio rerio*). The used doses were all below the median lethal dose (LD_{50}) and caused no significant difference in survival, deformities, or hatching between exposed and control groups. Compared to controls, there were transient UVA and UVB exposure effects on heart rate, with dose dependent reductions at 50 hpf, and at 60 hpf for UVA only. The UVB exposure caused an increasing trend in reactive oxygen species (ROS) formation at the two highest doses, even though only significant at 120 hpf for the second highest dose. Both UVA and UVB caused an increasing trend in lipid peroxidation (LPO) at the highest doses tested at 72 hpf. Furthermore, UVA exposure led to significant reductions in larval movement following exposure to the two highest doses of UVA, i.e., reduction in the time spent active and the total distance moved compared to control at 100 hpf, while no effect on the swimming speed was observed. The lowest dose of UVA had no effect on behaviour. In contrast, the highest dose of UVB led to a possible increase in the time spent active and a slower average swimming speed although these effects were not significant ($p = 0.07$). The obtained results show that UV doses below LD_{50} levels are able to cause changes in the behaviour and physiological parameters of zebrafish larvae, as well as oxidative stress in the form of ROS formation and LPO. Further testing is necessary to assess how this type of radiation and the effects observed could affect fish population dynamics.

1. Introduction

Ultraviolet light is ubiquitously present in the environment and classified into three categories: UVA (400–315 nm), UVB (315–280 nm), and UVC (280–100 nm), which is absorbed by the ozone layer and does not occur as part of the solar spectrum reaching the troposphere. The depletion of the ozone layer and climate change together are increasing the exposure of aquatic organisms to increasing levels of UVB and UVA radiation (Bais et al., 2018). It has been proposed that exposure to an altered UV regime can potentially cause differences in behavioural responses and possibly influence the level of biodiversity and food web dynamics in aquatic ecosystems (Bais et al., 2018).

Most studied aquatic organisms, particularly those inhabiting shallow aquatic environments, show susceptibility to the detrimental effects of UV radiation exposure (Häder et al., 2007). In general, it has been reported that fish spawning in shallow waters are most susceptible to the biologically damaging effects of UV radiation due to exposure of the vulnerable early larval stages, at a time when extensive DNA replication and organogenesis is taking place (Béland et al., 1999; Hunter et al., 1979). In sufficient doses (i.e. a longer exposure time), UV radiation can impair embryonic development in fish (Andrade et al., 2017; Fujimoto et al., 2007), and additionally it was found that zebrafish embryos at the gastrulation stage (starting from 5.25 h post fertilization (hpf)), were more tolerant to UV radiation compared to later developmental stages (Dong et al., 2007). Further, it was shown that even UVC radiation, at a wavelength outside the solar spectrum could inflict severe biological damage, whereby hindering the embryonic

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development in zebrafish (*Danio rerio*) via impairment of epiboly in the earliest post-zygotic stages (Strähle and Jesuthasan, 1993).

Moreover, UV radiation in sufficient doses can initiate a series of redox reactions to generate reactive oxygen species (ROS), which cause oxidative stress to cells and tissues during irradiation, but also as a result of disturbed cellular metabolic processes (Stańczyk et al., 2005). Although the exposure effects on oxidative stress parameters in larval fish have been reported after chronic UV irradiation during several days (Lesser et al., 2001; Mekkawy et al., 2010), it is less known whether these effects are persisting at later developmental stages.

In addition to the potential of UV radiation to induce oxidative stress, previous studies have shown that UVB exposure also caused differences in physiological and behavioural responses in fish larvae (Icoglu Aksakal and Ciltas, 2018), which are key life fitness traits essential for the growth and survival. Alterations in these responses would have severe consequences for the survival of these vulnerable early life-history stages. For example, an impairment of avoidance behaviour was demonstrated after exposure to environmentally relevant doses of UVB in cod (*Gadus morhua*) larvae (Fukunishi et al., 2012). In an earlier study, Alemanni et al. (2003) investigated the neurobehavioural effects of UVB exposure in juvenile rainbow trout (*Oncorhynchus mykiss*). These authors observed that irradiation with UVB from fluorescent tubes irreversibly increased trout O_2-consumption by individual fish. Further, rapid tail and fin movement as well as rapid and erratic displacements were observed at doses that caused changes in the O_2 consumption. In another study, Häkkinen et al. (2004) reported that exposure of newly fertilized pike (*Esox lucius*) eggs to UVB-doses similar to one daily erythema weighted ambient dose in Finland in May (0.27 J/cm^2, solar radiant exposure weighted by an action spectrum), resulted in neurobehavioural disorders such as inability to swim straight, circular movement and eventual mortality. However, to date insufficient data is available on the potential persistence of deleterious effects of UV irradiation during early life stages prior to hatching in fish.

The objective of this study was to investigate whether zebrafish sublethal UVA and UVB exposures during a vulnerable early life stage can cause persisting changes in physiological, oxidative stress parameters and lead to locomotor behavioural changes later in life. For this purpose, the zebrafish was selected as a model organism as it is a well-known model for developmental and behavioural toxicity assessment following environmental toxicant exposures (Ton et al., 2006; Parng et al., 2007; Selderslaghs et al., 2010; Colwill and Creton, 2011; Tierney, 2011). The doses used in this study correspond to a typical mid-summer, midday and clear sky average outdoor exposure in Oslo (60°N) of 10 and 150 min of UVB and UVA, respectively. Zebrafish from the late blastula to early gastrula stages (4.5–5.5 hpf), when the cell fate specification onset takes place (Kimmel et al., 1995; Montero et al., 2005) were used for the exposure studies. In addition to changes in larval behaviour, changes in heart rate as well as changes in oxidative stress were assessed.

2. Materials and methods

2.1. Fish husbandry

The study was performed at The Norwegian Zebrafish Platform of the Norwegian University of Life Sciences, Oslo, Norway. The unit is licensed by the Norwegian Animal Research Authority (NARA) (www.mattilsynet.no) and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (www.aaalac.org). The study was carried out under the regulations approved by the unit's animal ethics committee (Institutional Animal Care and Use Committee/ IACUC) following Norwegian laws and regulations controlling experiments and procedures on live animals in Norway. AB wild-type zebrafish were maintained at 28 °C under a 14:10 light/dark photoperiod. Adult care and breeding was in accordance with the local protocols previously described in Hurem et al. (2017). To generate embryos, adults were placed in spawning tanks in the afternoon, and the fish were spawned following the cessation of light (08:00) the next day, and the embryos collected (09:00) and maintained in sterile embryo media (60 µg/mL Instant Ocean® sea salts) until the time of exposure.

2.2. Ethical statement

All animal experiments in this study were performed in accordance with the Norwegian Animal Protection Act (implemented EU Directive 2010/63/EU) and larvae were euthanized at 120 hpf using an overdose of Tricaine (MS-222, Sigma Aldrich), followed by rapid freezing at (-70 °C).

2.3. UV exposure and embryo toxicity

Embryos between the late blastula (4.5 hpf) and early gastrula (5.5 hpf) stage of development were used for the UVA and UVB exposures (Table 1). All exposures were performed in polystyrene 50 × 9 mm Petri dishes (VWR, Radnor, PA, U.S) without the lid with 10 embryos in a 1 mL volume. Radiation exposure was performed using a modified exposure unit (Polylux PT, Dreve-Dentamid, Unna, Germany) consisting of three 9 W PL 12 UVB lamps (Philips, Eindhoven, The Netherlands) or three UVA-lamps, Osram GmbH DULUX S BL UVA 9 W/78. In order to remove UV with shorter wavelengths than 280 nm a filter material consisting of 5 mm Poly-Methyl-Methacrylate (Atoglas, Altuglas International) was placed in front of the exposure unit. The transmission of the filter was 100% for wavelengths above 300 nm. During irradiance measurement of the UVB-lamps, the filter was placed between the lamp and the detector to account for any absorption or light scatter in the material. The samples to be irradiated with UVB were placed 10 cm from the exposure unit. The UVA irradiation was performed with two exposure units placed on top of each other in a "sandwich" configuration with Petri dishes placed on a plate made of Atoglas in the gap between the exposure units. Thereby the dishes transparent to UVA were irradiated from both sides. The irradiance at the level of the dishes was estimated by adding the upward and downward fluxes. The spectrum and irradiance were determined by a scanning spectral radiometer (Bentham, UK, DTM 300 with a fibre optic light guide and cosine adapted diffuser D7). Constancy of the irradiance values was routinely performed with a Solar Light Co, PMA2100 (Philadelphia, USA) radiometer with appropriate detectors. The irradiance levels were 10.4 mW/cm^2 and 0.42 mW/cm^2 in UVA and UVB, respectively. The controls for UVB and UVA embryos were kept at room temperature (22 °C) during irradiation.

In order to determine the LD_{50}, 40 embryos distributed in 4 wells (10 embryos/ well) of a 12-well plate (Nunc™, Thermo-Fischer Scientific) were irradiated at approximately 5 hpf over the whole dose range. The number surviving a certain dose was scored at 48 hpf and expressed as surviving fraction relative to an unexposed control. The LD_{50} was found by linear extrapolation of data from 4 to 5 independent experiments (Table 1A). The subsequent lower UVA and UVB doses for the behaviour studies were chosen from the LD_{50} estimation. In order to determine the toxic effects of the used lower doses of UVA and UVB radiation exposure on the survival and development of the embryos and

Table 1
Doses for zebrafish UVA and UVB exposure experiments, group denotations and comparison to LD_{50}.

UVA exposure, 10.4 mW/cm^2			UVB exposure, 0.42 mW/cm^2		
Group	Exposure time (s)	Dose (J/cm^2), (% of LD_{50})	Group	Exposure time (s)	Dose (J/cm^2), (% of LD_{50})
Control	0	0	Control	0	0
UVA 1	900	9.4, (17%)	UVB 1	30	0.013, (13%)
UVA 2	1800	18.7, (34%)	UVB 2	60	0.025, (25%)
UVA 3	3600	37.4, (68%)	UVB 3	180	0.076, (76%)

larva, including the LD_{50}, the zebrafish embryo toxicity test (OECD/OCDE, 2013) was applied. Following exposure, embryos were incubated in 96 well plates (Nunc™, Thermo-Fischer Scientific) until 96 hpf. The survival, occurrence of deformities, and the median hatching time (HT_{50}) were assessed in embryonic and larval zebrafish exposed to doses lower than the LD_{50} presented in Table 1. In addition, body length was assessed at 72 hpf using a stereomicroscope in 20–30 replicate larvae without deformities per each exposure dose.

2.4. Heart rate

In order to determine the effects of UVA and UVB radiation exposure on the metabolism, the heart rate was assessed at 50 and 60 hpf using a light microscope and counted as the number of heart beats in a 15 s period. Eight to ten larvae/group were scored for each biological replicate (n = 38–53/group). For UVB, one biological replicate was missing, therefore an additional 8–10 larvae were analysed within the subsequent biological replicate.

2.5. Oxidative stress

2.5.1. ROS formation

Intracellular ROS production was determined in zebrafish after UV irradiation using the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA, Invitrogen, Molecular Probes Inc., Eugene, OR, USA) and according to the method described in Hurem et al. (2017). Briefly, embryos were individually collected and incubated in a 96-well black microplate (Corning Costar, Cambridge, MA, USA) for 1.5 h with H_2DCFDA, with 20–24 replicate embryos per exposure group at 70 hpf. Fluorescence was recorded at approximately 72 and 120 hpf in mean relative fluorescence units (RFU) using the Cytation 3 Cell Imaging Multi-Mode Reader (Biotek, Winooski Vermont, USA) and analysed using Gen5 Microplate Reader and Imager Software (Biotek, Winooski Vermont, USA). Natural fluorescence of irradiated egg water in combination with the probes (without presence of embryos) for each dose rate and the resulting fluorescence subtracted, including a positive control (1% H_2O_2) were also analysed. The relative fluorescence obtained for each exposure group was expressed as fold induction comparative to the control.

2.5.2. Lipid peroxidation

The lipid peroxidation was assessed by two methods. First, the probe C11-BODIPY$^{581/591}$ was used for measuring LPO in zebrafish larvae in a time-dependent manner. This probe is a fatty acid analogue with specific fluorescence properties, which can easily enter the lipid bilayer and be subject to oxidation by oxyl-radicals together with the endogenous fatty acids, once inside the cellular membrane (Drummen et al., 2002). Similarly to ROS formation, exposed embryos and controls were individually collected and incubated in a 96-well black microplate (Corning Costar, Cambridge, MA, USA) for 2 h with C11-BODIPY$^{581/591}$ (final concentration 10 μM), with 20–23 replicate embryos per exposure group at 70 hpf. Fluorescence was recorded by use of the same system as for ROS at 72, 96 and prior to 120 hpf and the results expressed as fold induction comparative to the control.

Lipid peroxidation was also determined in 72 hpf larvae by measurement of malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) concentrations upon decomposition by polyunsaturated fatty acid peroxides, following the method by Erdelmeier et al. (1998), previously described in zebrafish larvae (Hurem et al., 2017). Here, 15 zebrafish larvae were pooled per sample in triplicate biological samples per dose, with exception of duplicates in UVA2 and UVB3 (where an additional technical replicate was used).

2.6. Behavioural testing

For the behavioural analyses, immediately following exposure, individual embryos were placed in the wells of square 96 well plates (#7701-1651, Whatman, USA) with 500 μL of media and placed inside an incubator set to 28 °C with a 14:10 day/night cycle. UVA and UVB treated embryos were transferred to separate plates. For the first three biological replicates, the control and all 3 UVA and UVB doses were equally represented across two 96 well plates (n = 24/dose/plate), whereas for the final three biological replicates the control and all 3 UVA and UVB doses were equally represented across only one 96 well plate (n = 24/dose). The locomotor activity (LMR) of the larvae (total 139–143 larvae from 6 experiments) was visualized over a set time interval. Behavioural tests were conducted using a ViewPoint® Zebrabox system and the accompanying video tracking software (ViewPoint Life Sciences, Lyon, France), which is a high-throughput image analysis system that can visualize and quantify the zebrafish behavioural response. Behavioural screening was undertaken at 100 hpf. This corresponds to tests beginning 330 min (13:00) and 390 (14:00) minutes after the cessation of light (07:30) in the incubator for UVA and UVB, respectively. Larval behaviour, including the cumulative distance travelled and the time spent active per minute, were simultaneously measured for all larvae on a plate during a 50 min simulated light-dark-light cycle, consisting of 20 min of light, 20 min of darkness, and final 10 min of light. The average swimming speed was calculated by dividing the cumulated distance travelled with the total time spent active. The light level was set to 100% on the ViewPoint software. The larval activity was tracked during the dark period. After the behavioural test, the larvae were inspected with a stereo microscope to identify dead or deformed larvae.

2.7. Statistical analysis

After evaluating and arranging the data in Excel, the differences in general toxicity, heart rate and LPO between exposure groups were analysed using a one-way ANOVA and Tukey's multiple comparison tests (GraphPad Prism 7 Software Inc., La Jolla, CA, USA). Differences between dose and time were compared for ROS production using a Two-way ANOVA followed by the post-hoc Tukey test (GraphPad Prism 7 Software Inc., La Jolla, CA, USA). For behavioural analyses, data were transferred to R version 2.15.0 (R Development Core Team, http://www.r-project.org). Dead and deformed larvae were excluded from behavioural analyses. Only the cumulative data from the 20 min dark period of the test were used, as movement was minimal during the lighted periods. Linear mixed effect (LME) models were used within the "nlme" package of R to assess behaviour. The dependent variable was either the cumulative time spent active, the cumulative distance travelled, or average speed (calculated as the cumulated distance travelled/cumulated time spent active), with dose as a categorical independent variable, and replicate as a random effect. For all models, examination of the residual plots verified that no systematic patterns occurred in the errors (e.g. q-q plots). To assess individual doses to the controls, we used the contrast results provided within R. Significance in all tests was assigned at $p \le 0.05$.

3. Results

3.1. Developmental effects and heart rate

Analyses of mortality, deformities or hatching at 48, 72 and 96 hpf between controls and the exposed groups using the doses below LD_{50} showed no significant differences compared to controls and were generally below 10% (Tables 2A and 3A). There was no difference in mortality between 48 hpf and later time points. Additionally, there was no difference in body length at 72 hpf between exposed and control larvae (Table 2A).

UVA exposure significantly decreased the heart rate at 50 hpf in all exposed groups compared to controls, while at 60 hpf, the decrease remained significant only in the 18.7 and 37.4 J/cm^2 UVA doses

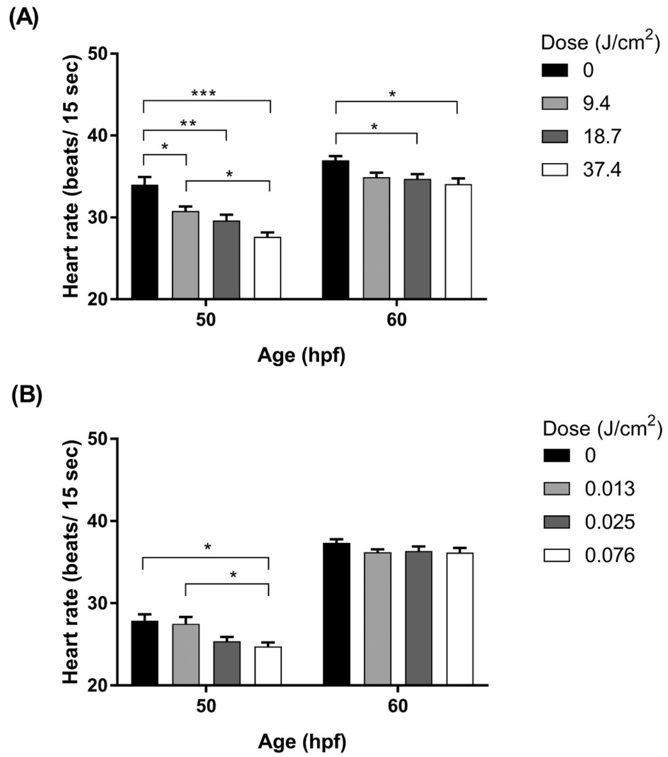

Fig. 1. Heart rate measured at 50 and 60 hpf in zebrafish exposed to sub-lethal UV radiation. Data presented as mean ± SEM. (One way ANOVA, $p < 0.006$). Significant difference between groups denoted with asterisks: (*) $p < 0.05$, (**) $p < 0.001$, (***) $p < 0.0001$ according to Tukey's test. **(A)** UVA exposure. **(B)** UVB exposure.

($p = 0.04$ and $p = 0.003$, respectively) (Fig. 1A). The results of UVB exposure showed a significant decrease in mean heart rate at the highest UVB dose compared to the controls ($p < 0.01$) at 50 hpf, while no significant differences were observed in the two lower UVB doses compared to controls (Fig. 1B). By 60 hpf, no differences in heart rate were found between the UVB exposed and control groups.

3.2. Oxidative stress

To assess the potential of UV radiation to generate ROS in zebrafish, the time dependent formation of ROS using a fluorescent probe was measured in all exposure groups. The results showed that for UVA both time and dose were significant for the differences seen in exposed larvae ($p < 0.0001$ and $p = 0.0025$, respectively). No clear pattern of increasing ROS formation was observed in the larvae after UVA exposure (Fig. 2A), while a trend of increasing ROS formation at the two highest UVB-doses was observed (Fig. 2B), although significantly increased only in the second highest dose at 120 hpf. Two-way ANOVA also showed that both time and dose affected ROS formation significantly for the UVB exposed groups ($p = 0.0006$ and $p < 0.0001$, respectively), and that their interaction was also significant ($p < 0.0001$) (Table 4A).

The formation of oxyl-radicals (HO·, ROO·, RO· and peroxynitrite) responsible for lipid oxidation was assessed by the fluorescent dye C11-BODIPY$^{581/591}$ in zebrafish larvae exposed to UVA and UVB. Results showed a small decrease in LPO after 72 hpf in the two highest UVA-doses when compared to the control (Fig. 3A), while no LPO was detected after 96 hpf. Additionally, no formation of oxyl-radicals was detected in larvae exposed to UVB (Fig. 3B).

On the other hand, the end-products of LPO, MDA and 4-HNE were determined at 72 hpf, where an increase (1.9-fold) in the highest dose was detected after the UVA exposure (Fig. 4A). In the UVB exposure, the highest dose demonstrated a non-significant increase in LPO compared to control, while the lowest UVB dose caused a significantly decreased LPO compared to control (Fig. 4B). Therefore, in both wavelength regions, a dose dependent increasing trend in LPO was observed.

3.3. Behaviour

Analysis of the locomotor activity (LMR) assay data showed that exposure to the highest and second highest dose of UVA significantly reduced the time spent active ($p = 0.02$ and $p = 0.04$, respectively) (Fig. 5A), while the highest dose also decreased the total distance moved compared to controls ($p = 0.03$), but had no effect on swimming speed. The lowest dose of UVA had no effect on behaviour. Exposure to the highest dose of UVB led to an increase in the time spent active (Fig. 5B), but a slower average swimming speed although these effects were not significant ($p = 0.07$). Neither of these tendencies were observed at lower UVB doses.

4. Discussion

This study examined the biological effects in zebrafish larvae following a short and low dose exposure to UVA and UVB radiation during a very sensitive life stage. The results demonstrated that the heart rate, oxidative stress parameters and the behaviour in fish aged 72–120 hpf may be persistently altered even at very low doses and that these alterations are wavelength and dose-dependent.

4.1. General toxicity and heart rate

UV radiation in high levels is able to induce acute toxicity in fish embryos and larvae. The LD_{50} determined following exposure to the mentioned UVA and UVB regimes confirmed that the doses used in this study are below the acute toxic levels. Comparable to the present results, Icoglu Aksakal and Ciltas (2018) reported effects on different parameters in zebrafish, whereby a mortality of 20% was observed at 24 hpf in embryos exposed to 0.1 J/cm^2 during a 3 h period in the blastula stage of development. Dong et al. (2007) used doses and dose rates higher than in the present study, but with different spectra, with LD_{50} about 20x higher in UVB and about 10x higher in UVA, whereby the segmentation stage (12–24 hpf) was more sensitive than the mid-blastula stage. Banerjee and Leptin (2014) exposed zebrafish embryos to lamps with wavelength around 320 nm, i.e., between UVA and UVB. The dose inducing close to 50% embryo mortality at 24 hpf was 0.024 J/cm^2, which is lower than here, but in accordance with the data of Dong et al. (2007), who found that mortality after UVB was higher at 24 hpf than at 3 hpf.

In order to study the behavioural effects and other effects not induced by acute toxicity, the used doses for late blastula to early gastrula (4.5–5.5 hpf) embryo exposure to UVA and UVB radiation corresponded to maximum 68% and 76% of the LD_{50}, respectively. Correspondingly, no differences were observed in development, hatching and body length at later stages of development from these exposures. Deformities included spinal aberrations, yolk sac or cardiac oedema, aberrations in pigmentation, and loss of equilibrium, but they were not statistically significant (Table 2A). However, the heart rate was significantly decreased compared to control after exposure to both UVA and UVB radiation at 50 hpf. This difference persisted until 60 hpf in the two highest doses of UVA compared to control groups, while in UVB no differences were observed at this stage. Together these results indicate that heart rate is a very sensitive endpoint susceptible to change after exposure to sub-lethal UV radiation in fish larvae, but that it also might be a temporary effect in the lower doses. In addition, the lowered heart rate may be connected to changes in metabolism and/or other physiological parameters, such as oxidative stress, as reported by Icoglu Aksakal and Ciltas (2018). Embryos of Atlantic cod (*Gadus morhua*) exposed to UVB from the sun seemed to be less sensitive by a

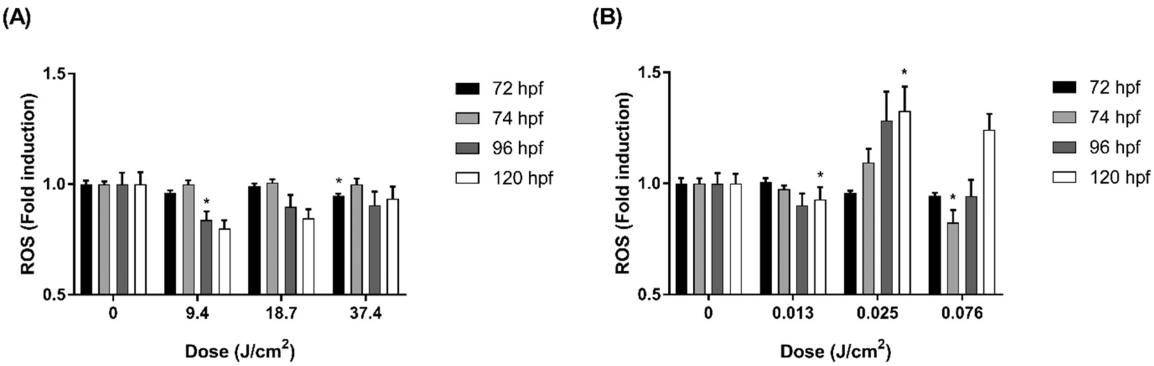

Fig. 2. ROS fold induction in zebrafish larvae from 72 hpf to 120 hpf exposed to UV radiation. Results presented as mean ± SEM. Significance in comparison to control denoted with asterisks (Two-way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$). (A) UVA exposure. (B) UVB exposure.

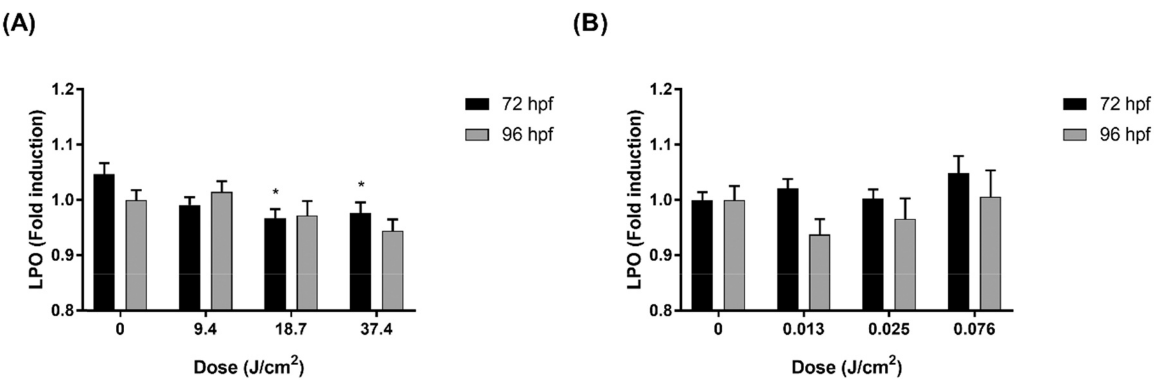

Fig. 3. Formation of oxyl-radicals in 72 hpf to 96 hpf zebrafish larvae exposed to UV radiation. Results presented as mean ± SEM. Significance in comparison to control denoted with asterisks (One way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$). (A) UVA exposure. (B) UVB exposure.

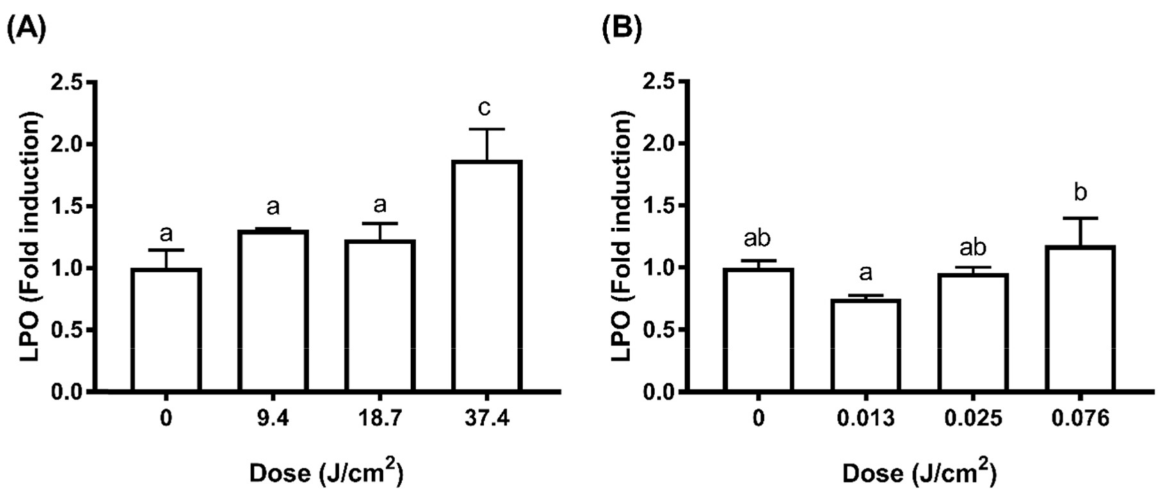

Fig. 4. Lipid peroxidation in 72 hpf zebrafish larvae after exposure to UV radiation. Results presented as mean ± SD. Significant difference between groups denoted with different letters, whereby letters shared by groups represent no significant difference (One way ANOVA, $p < 0.05$; Tukey's test, $p < 0.05$). **(A)** UVA exposure. **(B)** UVB exposure.

factor > 10 than zebrafish (Béland et al., 1999). It should be emphasized that the exposure conditions and the shape of the spectra varied widely in the studies cited above and direct comparisons of lethal doses are therefore impractical.

4.2. Oxidative stress

The use of fast and direct assays using fluorescent dyes has proven effective for the detection of oxidative stress caused by radiation at a whole-organism level, as previously seen in zebrafish larvae exposed to gamma radiation (Hurem et al., 2017). Results obtained using the H_2DCFDA probe showed that no significant formation of ROS was generated in zebrafish larvae exposed to UVA, contrary to what was expected. On the other hand, a more clear time and dose dependent increase was seen at the two highest UVB-doses, even though only significant at 120 hpf for the second highest dose. The reason for not observing an increase in ROS might be related to the time point of the ROS assessment. Zebrafish larvae exposed to both UV wavelengths could have undergone different chain reaction processes involved in the oxidative stress mechanism during exposure that could have accounted

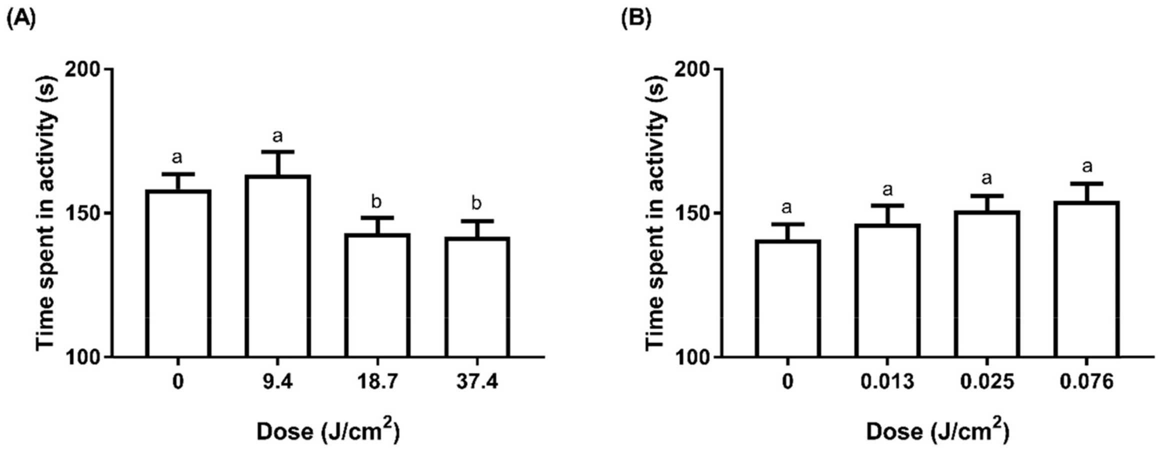

Fig. 5. Time spent in activity during the dark cycles of the locomotor assay measured in 100 hpf larval zebrafish after exposure to UV radiation. Data presented as mean ± SEM. Significance between groups denoted with different letters, whereby letters shared by groups represent no significant difference (linear mixed effect models $p \leq 0.05$, n = 139–143 larvae). **(A)** UVA exposure. (**B**) UVB exposure.

for a continuous formation and recycling of reactive species, which after 72 hpf were not present or not detected by H_2DCFDA. Another possibility for this lack of ROS formation in exposed larvae is the combined action of the antioxidant defence system that might have mediated the ROS formed during and after exposure. A study of chronic exposure of Atlantic cod (*Gadus morhua*) larvae to UVA and UVB radiation (0.001 and 0.006 J/cm^2 weighted dose), reported a significant increase in antioxidant enzyme activity after constant irradiation for 12 days, which is consistent with the previous notion.

LPO is a marker of oxidative damage and can potentially lead to cell death (Ayala et al., 2014; Livingstone, 2001; Gutteridge and Halliwell, 2010). In this study, no significant increase in LPO levels were detected using the C11-BODIPY$^{581/591}$ probe in zebrafish larvae exposed to both UVA and UVB (Fig. A1), even at the UVB doses where an increase in ROS levels was seen. This non-linearity between the formation of ROS and LPO levels detected in zebrafish larvae can be explained by the specificity of the two fluorescent probes towards different reactive species. The fluorescent probe H_2DCFDA has been shown to be reactive to a variety of ROS, particularly H_2O_2, HO$^·$, NO, ROO$^·$, O2– and peroxide-derived oxidants, while the C11-BODIPY$^{581/591}$ probe is triggered only in the presence of oxyl-radicals such as HO$^·$, ROO$^·$, RO$^·$ and ONOO$^-$ (Drummen et al., 2002). The absence of oxyl-radicals formation in exposed zebrafish can also be related to the formation of other end products of LPO, which are not detectable by this probe. In fact, the results obtained for LPO when measured as MDA and 4-HNE were more consistent to those obtained for ROS formation and showed significant damage at the highest UVA and UVB doses at 72 hpf. Although the ROS and LPO levels determined were not significantly different between the highest UVB dose and controls, the lowest UVB dose (0.013 J/cm^2) demonstrated a significant decrease in both parameters at 72 and 120 hpf, respectively. Together, these findings indicate that UVA at doses ≥ 37.4 J/cm^2 is potent enough to cause lipid peroxidation and consequently oxidative damage. It could be speculated that adaptive responses to the highest level of UVB exposure could lead to decreased LPO. This study has shown that oxidative stress parameters such as time dependent ROS formation and LPO can demonstrate changes persisting a longer time after early life UV exposure.

4.3. Behaviour

UV radiation levels in aquatic environments are strongly influenced by UV absorption in the water and sediments, and current levels have the potency to affect aquatic organisms and induce behavioural changes (Bais et al., 2017). Changes in behaviour may represent either compensatory and reversible adaptive responses in order to mitigate potential overt effects after perception of stress), such as reported in Atlantic cod after sea temperature changes (Alemanni et al., 2003; Freitas et al., 2015). They also may be irreversible effects of a toxicant on a behavioural mechanism or expression after toxicokinetic and toxicodynamic processes have started (Nellore, 2015) and are found to be an indicator of overall welfare (Martins et al., 2012). Some claim that behavioural changes might be pointing to neurodevelopmental toxicity of studied agents (Levin and Cerutti, 2009; Rihel and Schier, 2012).

Zebrafish larval behaviour was previously shown to be affected after exposure to various toxicants at the early embryonic stages (Nellore, 2015; Fraser et al., 2017). Here, behavioural changes resulting from a short duration early life exposure to UV were assessed 5 days post fertilization, and results showed that exposure to the two highest UVA doses resulted in a significant decrease in larval activity compared to the controls. As an example, a decrease in total movement can be an indicator of differences in anti-predator behaviour, concurring with earlier reports showing impaired escape behaviour in fish larvae after UV exposure (Fukunishi et al., 2012). The same exposure groups demonstrated a decrease in heart rate, which together with the decreased locomotor activity may be indicative of an overall lower metabolic activity as a consequence of UVA exposure. UVB exposure had no significant effect on larval activity. This result contradicts results obtained in studies of behaviour after exposure to environmentally relevant doses of UVB, whereby decreases in total movement in cod (*Gadus morhua*) larvae (Fukunishi et al., 2012) as well as behavioural differences in juvenile rainbow trout (*Oncorhynchus mykiss)* (Alemanni et al., 2003), were observed. Additionally, in pike eggs (*Esox lucius*), mortality occurred after exposure to UVB doses similar to one daily erythema weighted ambient dose in Finland in May, in addition to swimming disorders (about 0.27 J/cm^2) (Häkkinen et al., 2004), indicating that influence of UVB irradiation effects on the behaviour could have been a factor contributing to increased mortality.

Even though studies demonstrating an interaction of ROS production and behaviour in zebrafish larvae are lacking in the literature, at later developmental stages in zebrafish, an interaction of ROS production and movement was observed after chronic 3-h daily exposure to UVB radiation (Seebacher et al., 2016). In this study, the ROS formation was significantly decreased in the 37.4 J/cm^2 UVA dose at 72 hpf. The LPO in this group on the other hand, was increased at the same time point. In addition to the increased LPO in the highest UVA dose, the displayed decreased locomotor activity in these larvae might indicate that oxidative damage is affecting the behaviour.

5. Conclusion

Taken in account that climate change may increase exposure of aquatic organisms to increased UV radiation levels, it is important to assess how subtle changes in the UV regime might affect the physiological parameters and the behaviour as a key life fitness trait in aquatic populations. From the present findings, it can be concluded that an early life stage exposure to UVA and UVB radiation to sub-lethal and non-detrimental doses to zebrafish development can lower the metabolic activity in later stage embryos and fish larvae. However, depending on the exposure duration and wavelength, this effect persists only temporarily in the shortly exposed UVB groups. On the other hand, in the longer exposed UVA group (68% of the LD_{50}); lipid peroxidation persists for a longer time after the exposure, including the change in resting heart rate, while the total activity of fish larvae is reduced. The findings in this study show that even a very small change in the UV regime during a sensitive developmental stage can induce behavioural changes. Considering that these changes persist long after exposure to low doses of UV radiation during early life, they might have further implications for the fish population dynamics and warrant further studies.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.09.082

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